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Date: October 3, 1997
Docket No.: 2185-0208P-SP

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing is a patent application applied for on behalf of the inventor(s) according to the provisions of 37 CFR 1.41(c).

Inventor(s): KOSHIBA, Tomokazu

For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION THEREOF

Enclosed are:

X A specification consisting of 58 pages

___ ____ sheet(s) of _____ drawings

___ Certified copy of Priority Document(s)

X Executed Declaration in accordance with 37 CFR 1.64 will follow

___ A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27

X Preliminary Amendment

X Information Sheet

___ Information Disclosure Statement, PTO-1449 with reference(s)

___ Other _____

The filing fee has been calculated as shown below:

			LARGE ENTITY			SMALL ENTITY	
FOR	NO. FILED	NO. EXTRA	RATE	FEE		RATE	FEE
BASIC FEE	********** ********** **********	********** ********** **********	***** ***** *****	$790.00	or	**** **** ****	$395.00
TOTAL CLAIMS	58 - 20 =	38	x22 =$	836.00	or	x 11 = $	0.00
INDEPENDENT	2 - 3 =	0	x82 =$	0.00	or	x 41 = $	0.00
MULTIPLE DEPENDENT CLAIM PRESENTED yes			+270 =	$270.00	or	+135 = $	0.00
			TOTAL	$1,896.00		TOTAL $	0.00

X The application transmitted herewith is filed in accordance with 37 CFR 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed patent application together with the surcharge will be forwarded in due course.

X A check in the amount of $1,896.00 to cover the filing fee and recording fee (if applicable) is enclosed.

___ The Government Filing Fee will be paid at the time of completion of the filing requirement.

___ Please charge Deposit Account No. 02-2448 in the amount of $_____. A triplicate copy of this transmittal form is enclosed.

X Send Correspondence to: BIRCH, STEWART, KOLASCH & BIRCH, LLP
P. O. Box 747
Falls Church, Virginia 22040-0747

X If necessary, the Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 02-2448.

X Any additional filing fees required under 37 CFR 1.16.

X Any patent application processing fees under 37 CFR 1.17.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

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Document: 2185-0208P-SP

PATENT 2185-0208P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: T. KOSHIBA

Serial No.: NEW Group:

Filed: October 3, 1997 Examiner:

For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION THEREOF

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

October 3, 1997

Sir:

Applicants respectfully submit the following amendments and remarks in connection with the above-identified new application:

IN THE CLAIMS:

Claim 3, lines 1-2, delete "or 2".

REMARKS

It is submitted that the above amendment is made merely to delete the improper multiple dependency of the claim and does not constitute new matter. Favorable action on the above-identified application is respectfully requested.

Please charge any fees or credit any overpayment pursuant to 37 CFR 1.16 or 1.17 to Deposit Account No. 02-2448.

Respectfully submitted,

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2185-0208P-SP

IN THE U.S. PATENT AND TRADEMARK OFFICE

I N F O R M A T I O N S H E E T

Applicant: KOSHIBA, Tomokazu

Serial No.:

Filed: October 3, 1997

For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION THEREOF

Priority Claimed:

COUNTRY	DATE	NUMBER
Japan	10/04/96	08-283314

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The above information is submitted to advise the USPTO of all relevant facts in connection with the present application. A timely executed Declaration in accordance with 37 CFR 1.64 will follow.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

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DOCKET NO. 2185-0208P-SP

ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION THEREOF

FIELD OF THE INVENTION

The present invention relates to an aldehyde oxidase gene derived from a plant and utilization thereof.

It has been known that a natural plant growth hormone auxin alternatively IAA or indoleacetic acid is produced from tryptophane via indoleacetaldehyde followed by the action of an oxidase in higher plants. The hormone is deeply involved in various morphogenesis and environmental adaptation of a plant by its physiological activity and has significant effects on maturing by growth acceleration in general crop cultivation, improvement in yield and in quality by rooting acceleration in nursery plant production, increase in yield by growth acceleration of fruits in fruit vegetable cultivation, increase in added value by acceleration of flowering and elongation of life by prevention of defoliation or aging in ornamental plant cultivation. Therefore, there has been a strong demand for a method for artificially controlling the said enzyme for industry and particularly agricultural production.

Under these circumstances, the present inventors have successfully determined the total amino acid sequence and gene of the enzyme and completed the present invention.

Thus, the present invention provides:

1) An aldehyde oxidase gene which is a 4.4 Kbp gene obtainable from a plant and which encodes an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to a carboxylic acid (hereinafter, referred to as the gene of the present invention),

2) The aldehyde oxidase gene according to item 1), wherein the aldehyde compound is indoleacetaldehyde and the carboxylic acid is indoleacetic acid,

3) The aldehyde oxidase gene according to item 1 or 2 which is derived from a maize plant (*Zea mays L.*),

4) The aldehyde oxidase gene according to item 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1,

5) The aldehyde oxidase gene according to item 4 which has a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120),

6) The aldehyde oxidase gene according to item 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 3,

7) The aldehyde oxidase gene according to item 6 which has a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138),

8) A plasmid comprising the aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7,

9) A transformant transformed by introducing the plasmid according to item 8 into a host cell,

10) The transformant according to item 9, wherein the host cell is a microorganism,

11) The transformant according to item 9, wherein the host cell is a plant,

12) A process for constructing an expression plasmid which comprises ligating:

(1) a promoter capable of functioning in a plant cell,

(2) an aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7 and

(3) a terminator capable of functioning in a plant in a functional manner and in the said order described above,

13) An expression plasmid comprising:

(1) a promoter capable of functioning in a plant cell,

(2) an aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7 and

(3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above,

14) A process for controlling production of an aldehyde oxidase in a transformant which comprises introducing, into a host cell, an expression plasmid comprising:

(1) a promoter capable of functioning in a plant cell,

(2) an aldehyde oxidase gene and

(3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above to transform said host cell,

15) The process according to item 14, wherein the aldehyde oxidase gene is derived from a plant and the host cell is a plant, and

16) The process according to item 14, wherein the expression plasmid is the expression plasmid according to item 13.

EMBODIMENTS OF THE INVENTION

The present invention will be described in more detail.

The gene of the present invention comprises about 4.4 kbp nucleotide obtainable from a plant and is an aldehyde oxidase gene that encodes an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to generate a carboxylic acid. For example, it is capable of oxidizing indoleacetaldehyde to generate indoleacetic acid.

The gene of the present invention can be obtained from a plant, for example, maize or the like. The gene of the present invention and the enzyme as the translation product of it have an action of oxidizing an acetaldehyde compound to a carboxylic acid in a cell. Said enzyme may also act, for example, on benzaldehyde, butyraldehyde, protocatechualdehyde or the like as the substrate, in

addition to indolealdehyde. Of course, a single enzyme may act on plural compounds as substrates.

The gene of the present invention specifically includes, for example, a gene which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1 and a gene which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 3 as well as an equivalent of them. The expression "an equivalent of them" used herein means an aldehyde oxidase gene having a nucleotide sequence of an aldehyde oxidase gene that encodes an amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3 with a single nucleotide or plural nucleotides added, deleted or replaced, and refers to a DNA which is an analog having the same function. More particularly, this includes a gene having a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120) or a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138).

The gene of the present invention can be obtained by the following process.

For example, seeds of Golden Cross Bantam 70 (purchased from Sakata-no-tane), a maize cultivar, are subjected to a treatment for hastening of germination by immersing overnight in running tap water, subsequently seeded on a paper towel moistened with water and placed in red light (0.8 W/m^2)

under a condition of 25℃ for 2 days and then in the dark for 1 day to allow germination. Top portions of young sheaths grown to 1.0 - 1.5 cm from the obtained seedlings are excised under a green safety light, immediately frozen with liquid nitrogen and stored at -30℃ as samples for purification of enzymes and samples for extracting RNAs.

For purifying aldehyde oxidase from the frozen samples prepared in this manner, it is appropriate to use a method described in T. Koshiba et al., Plant Physiology, 1996, 110, 781 - 789.

In order to prevent decrease in activity of the enzyme and decomposition of the protein during procedures of extraction and purification, it is preferred to carry out all the treatments in the purification steps at a lower temperature of 2 - 4℃, as is ordinary manner in such procedures. First, 150 - 200 g of the frozen sample is taken as a material for one batch of purification. The material is mechanically crushed by a homogenizer or the like with addition of 400 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 g for 30 minutes. The supernatant is separated as a crude enzyme standard sample. From the crude enzyme standard sample, a fraction is obtained with 30 - 50% saturated ammonium sulfate, dialyzed against 20 mM Tris HCl buffer (pH 8.0) and centrifuged at 20,000 g for 20 minutes. The supernatant from centrifugation is passed over an

ion-exchange column (for example, DEAE TOYOPEARL 650 M, manufactured by Tosoh) and a fraction with an aldehyde oxidase activity is collected. Said fraction with the specific activity is subjected to chromatography with a hydrophobic column, a hydroxyapatite column and an ion-exchange column (for example, DEAE-5PM) in this order and purified until the fraction with aldehyde oxidase activity is detected as an almost single protein band by silver staining after electrophoresis.

According to the above described purification procedure, about 2,000 times purification, in terms of the amount of protein in the crude enzyme standard sample, is usually possible. It can be confirmed that the finally purified protein has a size of about 300 kD in molecular weight by the gel filtration column process. Further, it can be detected as a band having a size of about 150 kD in molecular weight by SDS polyacrylamide gel electrophoresis (SDS-PAGE), indicating that said enzyme forms a dimer.

In the above described fractionating process by column chromatography, effective collection of the fraction with aldehyde oxidase activity can be achieved making use of measurement of aldehyde oxidase activity in respective fractions. For this purpose, a method in which indoleacetaldehyde is added to the purified fraction as a substrate and the amount of produced indoleacetic acid is

determined by HPLC, for example, can be utilized. Precisely, 100 μl of reaction solution consisting of 5 - 50 μl of the purified fraction, 0.1 mM indoleacetaldehyde and 0.1 mM phosphate buffer (pH 7.4) is prepared. The solution is incubated at 30℃ for 30 minutes to effect the reaction and, immediately after, 8 μl of 1 N HCl, 5 μl of 2.0 M sodium hydrogen sulfite and 50 μl of methanol are added to the solution to quench the reaction. The reaction solution is centrifuged at 15,000 g for 5 minutes and 100 μl of the obtained supernatant is taken as a analytical sample for HPLC. By detecting absorption at 280 nm, indoleacetaldehyde as the substrate and indoleacetic acid as the reaction product can be quantitatively analyzed. It is effective to carry out HPLC with, for example, ODS C18 column and to elute with 20 - 50% linear gradient of methanol containing 0.1% acetic acid.

The protein obtained in this manner is partially digested and the digested peptide is analyzed to obtain a partial amino acid sequence information. Usually, the purified aldehyde oxidase sample is separated by SDS-PAGE and a protein band of 150 kD is collected by excision. The collected gel fragments are treated, for example, with Achromobacter Protease I (API) in the presence of 0.1% SDS and digested peptide fragments are extracted. This is loaded, for example, on a reverse phase HPLC accompanied by a pre-column of an anion exchanger (DEAE) to separate peptides

and recover them. The amino acid sequences are determined by a protein sequencer and parts of the samples are subjected to molecular weight determination by MALDI-TOF to check accuracy of the obtained amino acid sequence information.

Then, an oligo DNA expected to encode the amino acid sequence is synthesized on the basis of the obtained amino acid sequence information. Further, RT-PCR is conducted using a total RNA as a template to amplify cDNA partial fragment, which is then cloned into a plasmid vector.

For extraction of the total RNA, 7 g of the frozen sample, for example, is triturated in liquid nitrogen with a mortar and a pestle to form fine powders. After evaporating liquid nitrogen, RNA is extracted by the conventional manner, for example, using guanidine thiocyanate/cesium chloride process and the total RNA is collected from the extract by ethanol precipitation. By this procedure, usually 1 mg of the total RNA is obtained.

For amplification of cDNA, a reverse transcription reaction is carried out using, among synthetic oligo DNAs, one synthesized in antisense orientation as a primer and binding it to a transcription product of a target RNA contained in the total RNA. The reverse transcription reaction can be conducted using a commercially available reverse transcription PCR kit, for example, RNA-PCR kit (manufactured by Perkin-Elmer Cetus Instruments). Then, the

obtained reverse transcription product can be subjected again to PCR in which an oligo DNA synthesized in sense orientation is added to amplify cDNA fragment.

The obtained cDNA amplification fragment is purified and cloned into a plasmid vector. As the plasmid vector, for example, pCRII (manufactured by Invitrogen) can be used and cDNA amplification fragment can be cloned by transforming *E. coli* according to the conventional manner and screening transformants having an insert. The nucleotide sequence of the clone is determined using, for example, ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kits (manufactured by Applied Biosystems) on the obtained cDNA clone.

Sense and antisense primers for part of nucleotide sequence in cDNA partial fragment obtained in this manner can be synthesized and subjected to RACE to obtain cDNA fragments having terminals in 5'-orientation and 3'-orientation, respectively. A complete length cDNA can be obtained by ligating them and cloning into a plasmid vector. For the RACE, a commercially available Marathon cDNA Amplification Kit (manufactured by Clontech), for example, can be used.

The gene of the present invention can be utilized in the following manner.

For example, a host cell such as a microorganism, a plant or the like is transformed by introducing the gene of the

present invention to form a transformant.

In order to introduce and express the gene of the present invention in a plant cell, an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) a gene of the present invention (an aldehyde oxidase gene described in items 1 to 7 above) and (3) a terminator capable of functioning in a plant cell which are ligated in a functional manner in a plant cell and in the said order described above and introduced in a plant cell to transform said cell.

The expression "in a functional manner" used herein means that, when the constructed plasmid is introduced into a plant cell to transform it, the gene of the present invention is integrated under the control of a promoter such that the gene is normally transcribed/translated and have a function of expressing a protein in said plant cell.

The promoter capable of functioning in a plant cell includes, for example, T-DNA derived constitutive type promoters such as nopaline synthase gene (NOS) promoter, octopine synthase gene (OCS) promoter and the like, plant virus derived promoters such as cauliflower mosaic virus (CaMV) derived 18S and 35S promoters and the like, and inducible type promoters such as phenylalanine ammonialyase (PAL) gene promoter, chalcone synthase (CHS) gene promoter, pathogen-related (PR) gene promoter and the like. Further,

it includes other known plant promoters.

The terminator capable of functioning in a plant cell includes, for example, T-DNA derived constitutive type terminators such as nopaline synthase gene (NOS) terminator and the like, plant virus derived terminators such as garlic virus GV1, GV2 terminators and the like. Further, it includes other known plant terminators.

For transforming a plant cell by introducing such plasmid into a plant cell, the above described expression plasmid is introduced into a plant cell by any of conventional means such as *Agrobacterium* infection method (JP-B-2-58917 and JP-A-60-70080), electroporation method into protoplast (JP-A-60-251887 and JP-A-5-68575), particle gun method (JP-A-508316 and JP-A-63-258525) and the like, and a transformed plant cell can be obtained by selecting a plant cell into which the gene of the present invention is introduced. The transformed plant is obtained by regenerating a plant according to a conventional plant cell culturing process, for example, described in Uchimiya, Manual for Plant Gene Manipulation (Method for Producing Transgenic Plants), Published by Kodansha Scientific (ISBN 4-06-153515-7 C3045), 1990, pages 27 - 55.

Further, the present invention provides a process for controlling production of an aldehyde oxidase in a transformant which comprises introducing, into a host cell,

an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above to transform said host cell.

The promoter capable of functioning in a plant cell includes, for example, lacZ gene promoter of lactose operon in *E. coli*, alcohol dehydrogenase gene (ADH) promoter in yeast, Adenovirus major late (Ad.ML) promoter, early promoter of SV 40, Baculovirus promoter and the like. When the host is a plant, promoters capable of functioning in a plant as described above may also be included.

The terminator capable of functioning in a plant cell includes, for example, HIS terminator sequence in yeast, ADHI terminator, early splicing region of SV 40 and the like. When the host is a plant, terminators capable of functioning in a plant as described above may also be included.

The aldehyde oxidase gene may be any one insofar as it is a gene encoding an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to form a carboxylic acid. This includes, for example, aldehyde oxidase genes derived from plants and preferably the gene of the present invention (an aldehyde oxidase gene described in items 1 to 7 above).

Transformation of a host cell by introducing such plasmid into said host cell can be effected by a method

generally used in the field of genetic engineering.

When the host cell is a plant cell, it can be effected, for example, by a method generally used in the field of plant genetic engineering and the field of plant tissue cultivation as described above.

The transformation of a plant by introducing the gene of the present invention may bring about enhancement of generally known physiological action of auxin or supression of the same. For example, by enhancing the activity of auxin through a sense gene, elongation growth and differentiation to vascular bundle of the host cell can be accelerated resulting in growth acceleration of a plant and enhanced capacity of storing assimilation products. As a result, early maturing of crops, enlargement of harvest such as fruits and improvement in yield or quality can be expected and realized. To the contrary, by suppressing the activity of auxin through a sense gene, spindly growth of a plant is prevented and a plant capable of growing under improper environmental conditions such as insufficient insolation can be bred. Further, by adequately controlling growth, dwarfing of crops becomes possible and application, for example, to prevention of lodging of rice plants and shortening of cut flowers become possible. As a result, improvement in yield and quality can be expected.

Addition of hormone to the medium is generally essential

for aseptic cultivation of cells or tissue of a plant. When auxin activity in a plant is enhanced by introducing and expressing the gene of the present invention thereby increasing production of aldehyde oxidase in a transformant, said plant is expected to be in a state in which capacity of cell proliferation, differentiation and individual regeneration in the sterile culture is enhanced. Therefore, it is possible to create a so-called easily cultured strain and this is useful in the production of nursery plant of virus-free crops for which tissue culture-nucleotide mass culture is conducted and garden crops such as flower and ornamental plants.

EXAMPLES

The present invention will now be described in more detail by means of Examples. It is to be understood, however, that the scope of the present invention is not limited to these Examples.

Example 1 (Preparation of maize young sheath)

Seeds of Golden Cross Bantam 70 (purchased from Sakata-no-tane), a maize cultivar, were subjected to a treatment for hastening of germination by immersing overnight in running tap water, subsequently seeded on a paper towel moistened with water and placed in red light (0.8 W/m^2)

under a condition of 25℃ for 2 days and then in the dark for 1 day to allow germination. Top portions (1.0 - 1.5 cm) of young sheaths grown from the obtained seedlings to 2 - 3 cm were excised under a green safety light, immediately frozen with liquid nitrogen and stored at -30℃.

Example 2 (Preparation of aldehyde oxidase)

All the procedures in the following purification steps were conducted at a low temperature of 2 - 4℃.

First, about 200 g of the frozen sample prepared in Example 1 was taken as a material for one batch of purification. The material was mechanically crushed by a homogenizer with addition of 400 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 g for 30 minutes. The supernatant was separated as a crude enzyme standard sample. Subsequently, from the crude enzyme standard sample, a fraction was obtained with 30 - 50% saturated ammonium sulfate, dialyzed against 20 mM Tris HCl buffer (pH 8.0) and centrifuged at 20,000 g for 20 minutes. The supernatant from centrifugation was passed over an ion-exchange column (DEAE TOYOPEARL 650 M, manufactured by Tosoh) and a fraction with an aldehyde oxidase activity was collected on the basis of activity measurement conducted in a manner described below in Example 3. Said fraction with activity was subjected to chromatography with a hydrophobic column, a hydroxyapatite column and an

ion-exchange column (DEAE-5PM) in this order and purified until the fraction with aldehyde oxidase activity was detected as an almost single protein band by silver staining on electrophoresis.

By the above described purification procedure, about 0.09 mg of protein was recovered from 1,873 mg of protein in the crude enzyme standard sample, and ratio of enzyme activity for aldehyde oxidase to the original was 1,950 times. It was confirmed that the finally purified protein had a size of about 300 kD in molecular weight by the gel filtration column process. Further, it was detected as a band having a size of about 150 kD in molecular weight by SDS polyacrylamide gel electrophoresis (SDS-PAGE), indicating that said enzyme formed a dimer.

Example 3 (Method for measuring aldehyde oxidase activity)

Measurement of aldehyde oxidase activity in the respective purified fractions described in Example 2 was carried out by a method in which indoleacetaldehyde was added to the purified fraction as a substrate and the amount of produced indoleacetic acid (IAA) was determined by HPLC. The reaction was carried out with 100 μl of reaction solution consisting of 5 - 50 μl of the purified fraction, 0.1 mM indoleacetaldehyde and 0.1 mM phosphate buffer (pH 7.4). The solution was incubated at 30℃ for 30 minutes and, immediately

after, 8 μl of 1 N HCl, 5 μl of 2.0 M sodium hydrogen sulfite and 50 μl of methanol were added to the solution to quench the reaction. The reaction solution was centrifuged at 15,000 g for 5 minutes and 100 μl of the obtained supernatant was taken as a analytical sample for HPLC. By detecting absorption at 280 nm, indoleacetaldehyde and indoleacetic acid were quantitatively analyzed. HPLC was carried out with ODS C18 column and eluted with 20 - 50% linear gradient of methanol containing 0.1% acetic acid.

Example 4 (Peptide digestion of aldehyde oxidase: partial amino acid sequence)

The purified protein obtained in Example 2 was separated by SDS-PAGE and a protein band of 150 kD was collected by excision. The collected gel fragments were reacted with Achromobacter Protease I (API) in the presence of 0.1% SDS and digested peptide fragments were extracted. This was passed over a reverse phase HPLC accompanied by a pre-column of an anion exchanger (DEAE) to separate peptides, which were collected. The amino acid sequences were determined by a protein sequencer (ABI 477A).

As a result, the following 4 sequences were obtained as the partial amino acid sequences.

The first one was a sequence, shown below, having 18 amino acid residues:

Gln Val Asn Asp Val Pro Ile Ala Ala Ser Gly Asp Gly Trp Tyr His Pro Lys

and it was confirmed that the sequence corresponded to Nos. 235 to 252 residues in the amino acid sequence shown by SEQ ID NO: 1.

The second one was a sequence, shown below, having 16 amino acid residues:

Thr Asn Ser Asp Gly Leu Val Ile His Asp Gly Thr Trp Thr Tyr Lys

and it was confirmed that the sequence corresponded to 1,234 to 1,249 residues in the amino acid sequence shown by SEQ ID NO: 1 or to 1,226 to 1,241 residues in the amino acid sequence shown by SEQ ID NO: 3.

The third one was a sequence, shown below, having 20 amino acid residues:

Ser Ile Glu Glu Leu His Arg Leu Phe Asp Ser Ser Trp Phe Asp Asp Ser Ser Val Lys

and it was confirmed that the sequence corresponded to Nos. 253 to 272 residues in the amino acid sequence shown by SEQ ID NO: 1.

The fourth one was a sequence, shown below, having 21 amino acid residues:

Val Gly Ala Glu Ile Gln Ala Ser Gly Glu Ala Val Tyr Val Asp Asp Ile Pro Ala Pro Lys

and it was confirmed that the sequence corresponded to Nos. 591 to 611 residues in the amino acid sequence shown by SEQ ID NO: 1.

Parts of these digested peptide samples were subjected to molecular weight determination by MALDI-TOF to check accuracy of the obtained amino acid sequence.

Example 5 (Preparation of total RNA from maize young sheath and synthesis of cDNA)

In a manner similar to that in Example 1, seeds of maize were germinated and 7 g of top portions of the young sheath were collected from seedlings. These were frozen in 10 ml of liquid nitrogen and triturated with a mortar and a pestle to form fine powders. After evaporating liquid nitrogen, RNA was extracted by the conventional manner (guanidine thiocyanate/cesium chloride method) and 1 mg of the total RNA was collected from the extract by ethanol precipitation.

Example 6 (Preparation of an oligo DNA primer and RT-PCR)

A mixture of oligo DNAs expected to encode the partial amino acid sequence determined in Example 4 was synthesized in both sense and antisense orientation.

Specifically, as a nucleotide sequence expected from 8 amino acid residues: Val Ile His Asp Gly Thr Trp Thr in the partial amino acid sequence 2 described in Example 4, a 23-mer in antisense orientation: 5'-GTCCAIGTICC(AG)TC(AG)TGIATIAC-3' was synthesized.

Further, as a nucleotide sequence expected from 8 amino

acid residues: Gly Glu Ala Val Tyr Val Asp Asp in the partial amino acid sequence 4 described in Example 4, a 23-mer in sense orientation: 5'-GGIGA(AG)GCIGTITA(TC)GTIGA(TC)GA-3' was synthesized.

A reverse transcription reaction was carried out using, among them, one synthesized in antisense orientation as a primer and a commercially available reverse transcription PCR kit (RNA-PCR kit, manufactured by Perkin-Elmer Cetus Instruments). Then, the obtained reverse transcription product was subjected again to PCR in which an oligo DNA synthesized in sense orientation was added. As the result, amplification of cDNA fragment was confirmed.

Example 7 (Cloning of the PCR-amplified fragment into a vector and analysis of the structure)

The amplified cDNA fragment obtained in Example 6 was purified and cloned into a plasmid vector pCRII (manufactured by Invitrogen). Further, the nucleotide sequence of the insert in said plasmid vector was determined by 373A DNA Sequencer (manufactured by Applied Biosystems) using ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kits (manufactured by Applied Biosystems) and the structure of said cDNA fragment was determined. As a result, it was revealed that the cloned cDNA fragment contained 2 kinds having different structure, one corresponding to Nos. 1,839

to 3,785 nucleotides in the nucleotide sequence shown by SEQ ID NO: 2 and the other corresponding to Nos. 1,858 to 3,806 nucleotides in the nucleotide sequence shown by SEQ ID NO: 4.

Example 8 (Isolation of a complete length cDNA clone)

Based on the nucleotide sequence information obtained in Example 7, nucleotide sequences specific for said 2 cDNAs, respectively, were searched and oligo DNAs for the parts were synthesized in sense and antisense orientations.

Specifically, as the sense oligo DNAs corresponding to the nucleotide sequence shown by SEQ ID NO: 2, two kinds:
a 28-mer: 5'-GCTGGTCAAAATATTGGTGTCGTGATTG-3' (common), and
a 28-mer: 5'-GATTGCTGAAACACAAAGATATGCTAAT-3', and as the antisense oligo DNAs, four kinds:
a 27-mer: 5'-TGGCTGCAGATTTTCTGTGCTATACTC-3' (common),
a 27-mer: 5'-TGCTTTGCAGCCATATTAGCATATCTT-3',
a 24-mer: 5'-ACAGCCTTTTGGAAGCCACCTGGA-3', and
a 24-mer: 5'-ATCGGACTTGTTGTCGGCCTTGAC-3'
were synthesized.

Also, as the sense oligo DNAs corresponding to the nucleotide sequence shown by SEQ ID NO: 4, two kinds:
a 28-mer: 5'-GCTGGTCAAAATATTGGTGTCGTGATTG-3' (common), and
a 28-mer: 5'-GATTGCTCAAACACAGAAGTATGCCTAC-3', and as the antisense oligo DNAs, three kinds:

a 27-mer: 5'-TGGCTGCAGATTTTCTGTGCTATACTC-3' (common),

a 25-mer: 5'-CTTTGCCGCCATGTAGGCATACTTC-3', and

a 24-mer: 5'-TTCCACCTATGGTTGCAGTGTTCC-3'

were synthesized.

Using them as primers, RACE process was carried out with a commercially available Marathon cDNA Amplification Kit (manufactured by Clontech) to obtain cDNA fragments having terminals in 5'-orientation and 3'-orientation, respectively. Further, a complete length cDNA was obtained by ligating them and cloned into a plasmid vector pCRII (manufactured by Invitrogen).

Example 9 (Analysis of nucleotide sequence and determination of amino acid sequence of cDNA clones)

For two cDNA clones obtained in Example 8, analysis of nucleotide sequence was carried out with 373A DNA Sequencer (manufactured by Applied Biosystem) using ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kits, Dye Terminator Cycle Sequencing Kits (manufactured by Applied Biosystems). As a result, it was revealed that the genes of the present invention were cDNAs having 4,412 bp and 4,359 bp, respectively (see SEQ ID NOS: 2 and 4).

Further, based upon said nucleotide sequence, the total amino acid sequences encoded by the genes of the present invention were determined with GENETYX Gene Analysis

Software (manufactured by SDC, Software Development Co.). It was revealed that they were proteins having 1,358 and 1,349 amino acid residues, respectively (see SEQ ID NOS: 1 and 3).

Example 10 (Construction of aldehyde oxidase expression plasmid for direct introduction)

In order to allow expression of the gene of the present invention derived from maize by introducing in a plant cell, the following direct introduction expression vector for plant, for example, is constructed.

A GUS expression vector pBI221 (manufactured by Clontech) derived from pUC19 is digested by restriction enzymes SmaI and SacI (both being manufactured by Takara Shuzo) and 2.8 Kbp fraction is recovered removing GUS structural gene. The terminus is blunted with T4 DNA polymerase (manufactured by Takara Shuzo). Then, the terminus is treated for de-phosphorylation with bacterial alkaline phosphatase (manufactured by Takara Shuzo).

On the other hand, the complete length cDNA obtained in Example 8 is prepared for an insert gene and the terminus is blunted with T4 DNA polymerase in a similar manner. Afterwards, the both are ligated with T4 DNA ligase (DNA Ligation Kit Ver. 2, manufactured by Takara Shuzo) and used for transforming competent cells of *E. coli* HB101 strain

(manufactured by Takara Shuzo), from which Ampicillin resistant strains are selected. Among the recombinant plasmid amplified from the selected strains, clones in which a coding region for the aldehyde oxidase is inserted in normal orientation in relation to 35S promoter derived from cauliflower mosaic virus and the terminator derived from nopaline synthase and cloned in which said region is inserted in reverse orientation are selected and taken as expression vectors for direct introduction, respectively.

Example 11 (Construction of aldehyde oxidase expression plasmid for indirect introduction)

In order to allow expression of the aldehyde oxidase gene derived from maize by introducing in a plant cell, the following indirect introduction expression vector for plant, for example, is constructed.

In a manner similar to that in Example 10, the aldehyde oxidase gene of which the terminus is blunted is prepared for an insert gene. On the other hand, a GUS expression binary vector pBI121 (manufactured by Clontech) derived from pBIN19 is digested by restriction enzymes SmaI and SacI and a fraction is recovered removing GUS structural gene. The terminus is blunted in a similar manner and treated for de-phosphorylation. The both are ligated and used for transforming *E. coli*. The recombinant plasmid are selected

and taken as aldehyde oxidase expression vectors for indirect introduction. Further, the plasmid vectors are transferred to the strain *Agrobacterium tumefaciens* LBA4404 by the tri-parental method (GUS gene fusion system, manufactured by Clontech).

Example 12 (Creation of a transformed plant by introducing aldehyde oxidase expression plasmid; part 1)

The expression vectors for direct introduction obtainable in Example 10 are introduced by a particle gun into an aseptically cultured immature scutellum of rice plant according to a method described in Shimada et al., Ikushugaku Zasshi, 1994, 44 Supplement 1, 66, to obtain transformed rice plants. Similarly, they are introduced by a particle gun into an aseptically cultured immature scutellum of wheat plant according to a method described in Takumi et al., Ikushugaku Zasshi, 1995, 45 Supplement 1, 57, to obtain transformed wheat plants. Similarly, they are introduced by a particle gun into an aseptically cultured immature scutellum of barley plant according to a method described in Hagio et al., Ikushugaku Zasshi, 1994, 44 Supplement 1, 67, to obtain transformed barley plants. Similarly, they are introduced by particle gun into an adventitious embryo of maize according to a method described in M. E. Fromm et al., Bio/Technology, 1990, 8, 833 - 839, to obtain transformed

maize plants. Further, the expression vectors for direct introduction obtained in Example 10 are introduced by a particle gun into an adventitious embryo of soybean according to a method described in Japanese Patent Application Hei 3-291501 to obtain transformed soybean plants.

Example 13 (Creation of a transformed plant by introducing aldehyde oxidase expression plasmid; part 2)

The strains from *Agrobacterium tumefaciens* LBA4404 into which the aldehyde oxidase expression vectors for indirect introduction are introduced, obtainable in Example 11, are infected to an aseptically cultured leaf of tobacco by a method described in Uchimiya, Manual for Plant Gene Manipulation (Method for Producing Transgenic Plants), Published by Kodansha Scientific (ISBN4-06-153513-7), 1990, pages 27 - 33, to obtain transformed tobacco plants. Similarly, they are infected to a petiole of an aseptically cultured seedling of carrot by a method described in N. Pawlicki et al., Plant Cell, Tissue and Organ Culture, 1992, 31, 129 - 139, to obtain transformed carrot plants. Further, they are infected to a hypocotyl or cotyledon of an aseptically cultured seedling of Lotus corniculatus by a method described in Nagasawa et al., Ikushugaku Zasshi, 1995, 45 Supplement 1, 143, to obtain transformed Lotus corniculatus plants. Similarly, they are infected to an

aseptically cultured adventitious embryo of alfalfa by a method described in R. Desgagnes et al., Plant Cell, Tissue and Organ Culture, 1995, 42, 129 - 140, to obtain transformed alfalfa plants. Similarly, they are infected to an epycotyl or cotyledon of an aseptically cultured seedling of pea by a method described in J. Pounti-Kaerlas et al., Theoretical and Applied Genetics, 1990, 80, 246 - 252, to obtain transformed pea plants.

SEQ ID NO: 1

SEQUENCE LENGTH: 1,358

SEQUENCE TYPE: Amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays L.*)

STRAIN: cultivar: Golden Cross Bantam 70

SEQUENCE DESCRIPTION

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                20                  25                  30
Val Asn Gly Lys Arg Tyr Glu Ala Ala Gly Val Ala Pro Ser Thr Ser
        35                  40                  45
Leu Leu Glu Phe Leu Arg Thr Gln Thr Pro Val Arg Gly Pro Lys Leu
    50                  55                  60
Gly Cys Gly Glu Gly Gly Cys Gly Ala Cys Val Val Leu Val Ser Lys
65                  70                  75                  80
Tyr Asp Pro Ala Thr Asp Glu Val Thr Glu Phe Ser Ala Ser Ser Cys
                85                  90                  95
Leu Thr Leu Leu His Ser Val Asp Arg Cys Ser Val Thr Thr Ser Glu
            100                 105                 110
Gly Ile Gly Asn Thr Arg Asp Gly Tyr His Pro Val Gln Gln Arg Leu
        115                 120                 125
```

Ser Gly Phe His Ala Ser Gln Cys Gly Phe Cys Thr Pro Gly Met Cys

130 135 140

Met Ser Ile Phe Ser Ala Leu Val Lys Ala Asp Asn Lys Ser Asp Arg

145 150 155 160

Pro Asp Pro Pro Ala Gly Phe Ser Lys Ile Thr Thr Ser Glu Ala Glu

165 170 175

Lys Ala Val Ser Gly Asn Leu Cys Arg Cys Thr Gly Tyr Arg Pro Ile

180 185 190

Val Asp Thr Cys Lys Ser Phe Ala Ser Asp Val Asp Leu Glu Asp Leu

195 200 205

Gly Leu Asn Cys Phe Trp Lys Lys Gly Glu Glu Pro Ala Glu Val Ser

210 215 220

Arg Leu Pro Gly Tyr Asn Ser Gly Ala Val Cys Thr Phe Pro Glu Phe

225 230 235 240

Leu Lys Ser Glu Ile Lys Ser Thr Met Lys Gln Val Asn Asp Val Pro

245 250 255

Ile Ala Ala Ser Gly Asp Gly Trp Tyr His Pro Lys Ser Ile Glu Glu

260 265 270

Leu His Arg Leu Phe Asp Ser Ser Trp Phe Asp Asp Ser Ser Val Lys

275 280 285

Ile Val Ala Ser Asn Thr Gly Ser Gly Val Tyr Lys Asp Gln Asp Leu

290 295 300

Tyr Asp Lys Tyr Ile Asp Ile Lys Gly Ile Pro Glu Leu Ser Val Ile

305 310 315 320

Asn Lys Asn Asp Lys Ala Ile Glu Leu Gly Ser Val Val Ser Ile Ser

325 330 335

Lys Ala Ile Glu Val Leu Ser Asp Gly Asn Leu Val Phe Arg Lys Ile

340 345 350

Ala Asp His Leu Asn Lys Val Ala Ser Pro Phe Val Arg Asn Thr Ala

355 360 365

Thr Ile Gly Gly Asn Ile Met Met Ala Gln Arg Leu Pro Phe Glu Ser

370 375 380

Asp Val Ala Thr Val Leu Leu Ala Ala Gly Ser Thr Val Thr Val Gln

385 390 395 400

Val Ala Ser Lys Arg Leu Cys Phe Thr Leu Glu Glu Phe Leu Glu Gln

405 410 415

Pro Pro Cys Asp Ser Arg Thr Leu Leu Leu Ser Ile Phe Ile Pro Glu

420 425 430

Trp Gly Ser Asp Tyr Val Thr Phe Glu Thr Phe Arg Ala Ala Pro Arg

435 440 445

Pro Phe Gly Asn Ala Val Ser Tyr Val Asn Ser Ala Phe Leu Ala Arg

450 455 460

Thr Ser Gly Ser Leu Leu Ile Glu Asp Ile Cys Leu Ala Phe Gly Ala

465 470 475 480

Tyr Gly Val Asp His Ala Ile Arg Ala Lys Lys Val Glu Asp Phe Leu

485 490 495

Lys Gly Lys Ser Leu Ser Ser Phe Val Ile Leu Glu Ala Ile Lys Leu

500 505 510

Leu Lys Asp Thr Val Ser Pro Ser Glu Gly Thr Thr His His Glu Tyr

515 520 525

Arg Val Ser Leu Ala Val Ser Phe Leu Phe Ser Phe Leu Ser Ser Leu

530 535 540

Ala Asn Ser Ser Ser Ala Pro Ser Asn Ile Asp Thr Pro Asn Gly Ser

545 550 555 560

Tyr Thr His Glu Thr Gly Ser Asn Val Asp Ser Pro Glu Arg His Ile

565 570 575

Lys Val Asp Ser Asn Asp Leu Pro Ile Arg Ser Arg Gln Glu Met Val

580 585 590

Phe Ser Asp Glu Tyr Lys Pro Val Gly Lys Pro Ile Lys Lys Val Gly

595 600 605

Ala Glu Ile Gln Ala Ser Gly Glu Ala Val Tyr Val Asp Asp Ile Pro

610 615 620

Ala Pro Lys Asp Cys Leu Tyr Gly Ala Phe Ile Tyr Ser Thr His Pro

625 630 635 640

His Ala His Val Arg Ser Ile Asn Phe Lys Ser Ser Leu Ala Ser Gln

645 650 655

Lys Val Ile Thr Val Ile Thr Ala Lys Asp Ile Pro Ser Gly Gly Glu

660 665 670

Asn Ile Gly Ser Ser Phe Leu Met Gln Gly Glu Ala Leu Phe Ala Asp

675 680 685

Pro Ile Ala Glu Phe Ala Gly Gln Asn Ile Gly Val Val Ile Ala Glu

690 695 700

Thr Gln Arg Tyr Ala Asn Met Ala Ala Lys Gln Ala Val Val Glu Tyr

705 710 715 720

Ser Thr Glu Asn Leu Gln Pro Pro Ile Leu Thr Ile Glu Asp Ala Ile

725 730 735

Gln Arg Asn Ser Tyr Ile Gln Ile Pro Pro Phe Leu Ala Pro Lys Pro

740 745 750

Val Gly Asp Tyr Asn Lys Gly Met Ala Glu Ala Asp His Lys Ile Leu

755 760 765

Ser Ala Glu Val Lys Leu Glu Ser Gln Tyr Tyr Phe Tyr Met Glu Thr

770 775 780

Gln Ala Ala Leu Ala Ile Pro Asp Glu Asp Asn Cys Ile Thr Ile Tyr

785 790 795 800

Ser Ser Thr Gln Met Pro Glu Leu Thr Gln Asn Leu Ile Ala Arg Cys

805 810 815

Leu Gly Ile Pro Phe His Asn Val Arg Val Ile Ser Arg Arg Val Gly

820 825 830

Gly Gly Phe Gly Gly Lys Ala Met Lys Ala Thr His Thr Ala Cys Ala

835 840 845

Cys Ala Leu Ala Ala Phe Lys Leu Arg Arg Pro Val Arg Met Tyr Leu

850 855 860

Asp Arg Lys Thr Asp Met Ile Met Ala Gly Gly Arg His Pro Met Lys

865 870 875 880

Ala Lys Tyr Ser Val Gly Phe Lys Ser Asp Gly Lys Ile Thr Ala Leu

885 890 895

His Leu Asp Leu Gly Ile Asn Ala Gly Ile Ser Pro Asp Val Ser Pro

900 905 910

Leu Met Pro Arg Ala Ile Ile Gly Ala Leu Lys Lys Tyr Asn Trp Gly

915 920 925

Thr Leu Glu Phe Asp Thr Lys Val Cys Lys Thr Asn Val Ser Ser Lys

930 935 940

Ser Ala Met Arg Ala Pro Gly Asp Val Gln Gly Ser Phe Ile Ala Glu

945 950 955 960

Ala Ile Ile Glu His Val Ala Ser Ala Leu Ala Leu Asp Thr Asn Thr

965 970 975

Val Arg Arg Lys Asn Leu His Asp Phe Glu Ser Leu Glu Val Phe Tyr

980 985 990

Gly Glu Ser Ala Gly Glu Ala Ser Thr Tyr Ser Leu Val Ser Met Phe

995 1000 1005

Asp Lys Leu Ala Leu Ser Pro Glu Tyr Gln His Arg Ala Ala Met Ile

1010 1015 1020

Glu Gln Phe Asn Ser Ser Asn Lys Trp Lys Lys Arg Gly Ile Ser Cys

1025 1030 1035 1040

Val Pro Ala Thr Tyr Glu Val Asn Leu Arg Pro Thr Pro Gly Lys Val

1045 1050 1055

Ser Ile Met Asn Asp Gly Ser Ile Ala Val Glu Val Gly Gly Ile Glu

1060 1065 1070

Ile Gly Gln Gly Leu Trp Thr Lys Val Lys Gln Met Thr Ala Phe Gly

1075 1080 1085

Leu Gly Gln Leu Cys Pro Asp Gly Gly Glu Cys Leu Leu Asp Lys Val

1090 1095 1100

Arg Val Ile Gln Ala Asp Thr Leu Ser Leu Ile Gln Gly Gly Met Thr

1105 1110 1115 1120

Ala Gly Ser Thr Thr Ser Glu Thr Ser Cys Glu Thr Val Arg Gln Ser

1125 1130 1135

Cys Val Ala Leu Val Glu Lys Leu Asn Pro Ile Lys Glu Ser Leu Glu

1140 1145 1150

Ala Lys Ser Asn Thr Val Glu Trp Ser Ala Leu Ile Ala Gln Ala Ser

1155 1160 1165

Met Ala Ser Val Asn Leu Ser Ala Gln Pro Tyr Trp Thr Pro Asp Pro

1170 1175 1180

Ser Phe Lys Ser Tyr Leu Asn Tyr Gly Ala Gly Thr Ser Glu Val Glu

1185 1190 1195 1200

Val Asp Ile Leu Thr Gly Ala Thr Thr Ile Leu Arg Ser Asp Leu Val

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Lys Thr Asn Ser Asp Gly Leu Val Ile His Asp Gly Thr Trp Thr Tyr

1250 1255 1260

Lys Ile Pro Thr Val Asp Asn Ile Pro Lys Glu Phe Asn Val Glu Met

1265 1270 1275 1280

Phe Asn Ser Ala Pro Asp Lys Lys Arg Val Leu Ser Ser Lys Ala Ser

1285 1290 1295

Gly Glu Pro Pro Leu Val Leu Ala Thr Ser Val His Cys Ala Met Arg

1300 1305 1310

Glu Ala Ile Arg Ala Ala Arg Lys Glu Phe Ser Val Ser Thr Ser Pro

1315 1320 1325

Ala Lys Ser Ala Val Thr Phe Gln Met Asp Val Pro Ala Thr Met Pro

1330 1335 1340

Val Val Lys Glu Leu Cys Gly Leu Asp Val Val Glu Arg Tyr Leu Glu

1345 1350 1355

Asn Val Ser Ala Ala Ser Ala Gly Pro Asn Thr Ala Lys Ala

SEQ ID NO: 2

SEQUENCE LENGTH: 4,412

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays L.*)

STRAIN: cultivar: Golden Cross Bantam 70

FEATURES OF SEQUENCE:

KEY: CDS

LOCATION: 46..4120 (including termination codon)

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION

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GTG CTG TGT TGT GCT GTG CTG CGT GCT GTG GAG GGG GAG GAG GAG ATG     48

GGG AAG GAG GCA GGG GCA GCG GAG TCG TCG ACG GTG GTG CTG GCC GTC     96

AAC GGC AAG CGC TAC GAG GCG GCC GGC GTG GCT CCG TCC ACG TCG CTG    144

CTG GAG TTC CTC CGC ACC CAG ACG CCC GTC AGA GGC CCC AAG CTC GGC    192

TGC GGC GAA GGT GGC TGC GGT GCA TGC GTG GTC CTC GTC TCC AAG TAC    240

GAC CCG GCC ACG GAC GAG GTG ACC GAG TTC TCT GCC AGC TCC TGC CTG    288

ACG CTG CTC CAC AGC GTG GAC CGC TGC TCA GTG ACC ACC AGC GAG GGA    336

ATC GGC AAC ACC AGG GAT GGC TAC CAC CCC GTG CAG CAG CGC CTC TCC    384

GGC TTC CAC GCC TCG CAG TGC GGC TTC TGC ACA CCC GGC ATG TGC ATG    432

TCC ATC TTC TCC GCC CTT GTC AAG GCC GAC AAC AAG TCC GAT CGC CCG    480
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GAC CCT CCT GCT GGC TTC TCC AAG ATC ACT ACC TCG GAG GCA GAG AAG 528

GCT GTC TCG GGC AAC CTT TGT CGT TGC ACC GGA TAC AGA CCC ATT GTT 576

GAC ACC TGC AAA AGC TTT GCC TCT GAT GTT GAC CTC GAG GAC CTA GGC 624

CTC AAC TGT TTC TGG AAG AAG GGC GAA GAA CCT GCA GAA GTC AGC AGG 672

CTG CCG GGG TAC AAC AGC GGT GCC GTC TGC ACC TTT CCA GAG TTT CTC 720

AAA TCC GAA ATC AAG TCT ACT ATG AAG CAG GTG AAC GAT GTC CCC ATT 768

GCA GCC TCA GGT GAT GGC TGG TAC CAT CCT AAG AGC ATT GAA GAG CTT 816

CAC AGG TTG TTT GAT TCC AGC TGG TTT GAT GAC AGT TCT GTG AAG ATT 864

GTT GCT TCA AAC ACT GGG TCT GGA GTG TAC AAG GAT CAG GAC CTC TAC 912

GAC AAG TAC ATT GAC ATC AAA GGA ATC CCA GAG CTT TCA GTC ATC AAT 960

AAA AAC GAC AAA GCA ATT GAG CTT GGA TCA GTT GTG TCC ATC TCT AAA 1008

GCT ATT GAA GTG CTG TCA GAT GGA AAT TTG GTC TTC AGA AAG ATT GCT 1056

GAT CAC CTC AAC AAA GTG GCT TCA CCG TTT GTT CGG AAC ACT GCA ACC 1104

ATA GGA GGA AAC ATA ATG ATG GCA CAA AGG TTG CCA TTT GAA TCG GAT 1152

GTT GCA ACC GTG CTC CTA GCT GCG GGT TCG ACA GTC ACA GTC CAG GTG 1200

GCT TCC AAA AGG CTG TGC TTC ACT CTG GAG GAA TTC TTG GAA CAA CCT 1248

CCA TGT GAT TCT AGG ACC CTG CTG CTG AGC ATA TTT ATC CCA GAA TGG 1296

GGT TCA GAC TAT GTC ACC TTT GAG ACT TTC CGA GCC GCC CCA CGA CCA 1344

TTT GGA AAT GCT GTC TCT TAT GTA AAC TCT GCT TTC TTG GCA AGG ACA 1392

TCA GGC AGC CTT CTA ATT GAG GAT ATA TGC TTG GCA TTT GGT GCC TAC 1440

GGA GTC GAT CAT GCC ATC AGA GCT AAG AAG GTT GAA GAT TTC TTG AAG 1488

GGA AAA TCG CTG AGC TCA TTT GTG ATA CTT GAA GCA ATT AAA CTA CTC 1536

AAA GAT ACC GTT TCA CCA TCA GAA GGC ACT ACA CAT CAT GAA TAC AGG 1584

GTC AGC TTG GCT GTC AGT TTC TTG TTC AGT TTC TTA TCT TCC CTT GCC 1632

AAC AGT TCG AGT GCA CCA TCA AAT ATT GAT ACT CCC AAT GGG TCA TAT 1680

ACT CAT GAA ACT GGT AGC AAT GTG GAC TCA CCT GAG AGG CAT ATT AAG 1728

GTT GAC AGC AAT GAT TTG CCA ATT CGT TCA AGA CAA GAA ATG GTT TTC 1776

AGC GAT GAG TAC AAG CCT GTT GGC AAG CCG ATC AAG AAA GTC GGG GCA 1824

GAG ATC CAA GCA TCA GGG GAG GCT GTG TAC GTT GAT GAT ATC CCT GCT 1872

CCC AAG GAT TGC CTC TAT GGA GCA TTT ATC TAC AGC ACA CAT CCT CAT 1920

GCT CAT GTG AGA AGT ATC AAC TTC AAA TCA TCC TTG GCT TCA CAG AAG 1968

GTC ATC ACA GTT ATA ACC GCA AAG GAT ATT CCA AGC GGT GGA GAA AAT 2016

ATT GGA AGC AGC TTC CTG ATG CAA GGA GAA GCA CTA TTT GCA GAT CCA 2064

ATC GCT GAA TTT GCT GGT CAA AAT ATT GGT GTC GTG ATT GCT GAA ACA 2112

CAA AGA TAT GCT AAT ATG GCT GCA AAG CAA GCT GTT GTT GAG TAT AGC 2160

ACA GAA AAT CTG CAG CCA CCA ATT CTG ACA ATA GAA GAT GCC ATC CAA 2208

AGA AAC AGC TAC ATC CAA ATT CCC CCA TTT TTA GCT CCA AAG CCA GTT 2256

GGT GAC TAC AAC AAA GGG ATG GCT GAA GCA GAC CAC AAG ATT CTA TCA 2304

GCA GAG GTA AAA CTT GAA TCC CAG TAC TAC TTC TAC ATG GAA ACT CAA 2352

GCA GCA CTA GCG ATT CCT GAT GAA GAT AAC TGC ATA ACA ATC TAT TCC 2400

TCG ACA CAA ATG CCT GAG CTC ACA CAA AAT TTG ATA GCA AGG TGT CTT 2448

GGC ATT CCA TTT CAC AAT GTC CGT GTC ATC AGC AGA AGA GTA GGA GGA 2496

GGC TTT GGT GGA AAG GCA ATG AAA GCA ACG CAT ACT GCA TGT GCA TGT 2544

GCC CTT GCT GCC TTC AAG CTG CGG CGT CCA GTT AGG ATG TAC CTC GAT 2592

CGC AAG ACG GAC ATG ATA ATG GCT GGA GGG AGA CAT CCA ATG AAG GCG 2640

AAG TAC TCT GTT GGG TTC AAG TCA GAT GGC AAG ATC ACA GCC TTG CAC 2688

CTA GAT CTT GGA ATC AAT GCT GGA ATA TCA CCA GAT GTG AGT CCA TTG 2736

ATG CCA CGT GCT ATC ATA GGA GCT CTC AAA AAG TAC AAC TGG GGC ACT 2784

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GCA ATG CGA GCT CCT GGA GAT GTG CAG GGC TCT TTC ATC GCT GAA GCC 2880

ATC ATC GAG CAT GTT GCC TCA GCA CTC GCA CTA GAC ACT AAC ACC GTC 2928

AGG AGG AAG AAC CTT CAT GAT TTT GAA AGC CTT GAA GTT TTC TAT GGA 2976

GAA AGT GCA GGT GAA GCT TCT ACA TAC AGC CTG GTT TCC ATG TTT GAC 3024

AAG CTG GCC TTG TCT CCA GAA TAC CAG CAC AGG GCT GCA ATG ATT GAG 3072

CAG TTC AAT AGC AGC AAC AAA TGG AAG AAA CGC GGC ATT TCT TGT GTG 3120

CCA GCC ACT TAT GAG GTT AAT CTT CGA CCA ACT CCA GGC AAG GTG TCA 3168

ATC ATG AAT GAT GGT TCC ATC GCT GTC GAG GTT GGA GGA ATT GAG ATA 3216

GGT CAA GGA TTG TGG ACT AAA GTG AAG CAG ATG ACG GCC TTT GGA CTG 3264

GGA CAG CTG TGT CCT GAT GGT GGC GAA TGC CTT CTG GAC AAG GTT CGG 3312

GTT ATC CAG GCA GAC ACA TTA AGC CTG ATC CAA GGA GGT ATG ACT GCT 3360

GGG AGC ACC ACT TCT GAA ACT AGC TGT GAA ACA GTT CGG CAA TCT TGT 3408

GTT GCA CTG GTT GAG AAG CTG AAC CCT ATC AAG GAG AGT CTC GAA GCT 3456

AAG TCC AAC ACA GTG GAA TGG AGT GCC TTG ATT GCT CAG GCA AGC ATG 3504

GCG AGT GTG AAC CTA TCA GCA CAG CCG TAC TGG ACT CCT GAT CCA TCT 3552

TTC AAG AGC TAC TTG AAC TAC GGA GCT GGC ACC AGT GAG GTG GAA GTT 3600

GAT ATC CTA ACA GGA GCA ACC ACA ATT CTG CGA AGC GAC CTG GTG TAT 3648

GAC TGC GGG CAG AGC CTA AAC CCT GCT GTA GAC TTG GGC CAG ATC GAG 3696

GGC TGC TTT GTC CAA GGA ATA GGG TTC TTC ACG AAC GAG GAC TAC AAG 3744

ACG AAT TCC GAC GGG TTG GTC ATC CAC GAC GGC ACA TGG ACG TAC AAG 3792

ATC CCC ACG GTG GAT AAT ATC CCG AAG GAG TTC AAT GTT GAG ATG TTT 3840

AAC AGC GCC CCT GAC AAG AAG CGT GTC CTA TCT TCC AAA GCG TCG GGC 3888

GAG CCG CCG CTG GTT CTC GCA ACC TCG GTG CAC TGC GCG ATG AGG GAG 3936

GCC ATC AGG GCG GCG AGG AAG GAG TTC TCG GTC AGC ACC AGC CCC GCG 3984

AAA TCC GCC GTC ACA TTC CAG ATG GAC GTG CCG GCG ACG ATG CCT GTC 4032

GTC AAG GAG CTC TGC GGC CTC GAC GTC GTG GAG AGG TAC CTC GAG AAC 4080

GTG TCT GCC GCC AGT GCC GGC CCA AAC ACA GCG AAA GCA TAG ATC CAG 4128

CAG GCC TCA GGG TGC AGT CGG CGC ACT GCC AGA GAT GAT GTG TGC TGC 4176

CCT GAT GTA CAG ACA GTA CAG TAC AGA GGA GAG AGA ATT GGG GGA ACT 4224

CAG GAA CTG CGA GGA GCG ATG AAC AGT ATA TAG AGT GAA AAA TAA AAG 4272

TGC TTC GTA CTA ATA ATC ACT AGA AAA AAT TAT GCA CAT CTC CCA CGC 4320

ACT ACC GGC ACG ACT GTT GAA TAT TTT GTA AAA TAA GAT GTC ATA AGC 4368

TAT TTA TTT TCT GTA AAA AAA AAA AAA AAA AAA AAA AAA AAA AA 4412

SEQ ID NO: 3

SEQUENCE LENGTH: 1,349

SEQUENCE TYPE: Amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays L.*)

STRAIN: cultivar: Golden Cross Bantam 70

SEQUENCE DESCRIPTION

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Met Glu Met Gly Lys Ala Ala Ala Val Val Leu Ala Val Asn Gly Lys
                20                  25                  30
Arg Tyr Glu Ala Ala Gly Val Asp Pro Ser Thr Thr Leu Leu Glu Phe
        35                  40                  45
Leu Arg Thr His Thr Pro Val Arg Gly Pro Lys Leu Gly Cys Gly Glu
    50                  55                  60
Gly Gly Cys Gly Ala Cys Val Val Leu Val Ser Lys Tyr Asp Pro Ala
65                  70                  75                  80
Thr Asp Glu Val Thr Glu Phe Ser Ala Ser Ser Cys Leu Thr Leu Leu
                85                  90                  95
His Ser Val Asp Arg Cys Ser Val Thr Thr Ser Glu Gly Ile Gly Asn
            100                 105                 110
Thr Lys Asp Gly Tyr His Pro Val Gln Gln Arg Leu Ser Gly Phe His
        115                 120                 125
```

Ala Ser Gln Cys Gly Phe Cys Thr Pro Gly Met Cys Met Ser Ile Phe

130 135 140

Ser Ala Leu Val Lys Ala Asp Lys Ala Ala Asn Arg Pro Ala Pro Pro

145 150 155 160

Ala Gly Phe Ser Lys Leu Thr Ser Ser Glu Ala Glu Lys Ala Val Ser

165 170 175

Gly Asn Leu Cys Arg Cys Thr Gly Tyr Arg Pro Ile Val Asp Ala Cys

180 185 190

Lys Ser Phe Ala Ala Asp Val Asp Leu Glu Asp Leu Gly Leu Asn Cys

195 200 205

Phe Trp Lys Lys Gly Asp Glu Pro Ala Asp Val Ser Lys Leu Pro Gly

210 215 220

Tyr Asn Ser Gly Asp Val Cys Thr Phe Pro Asp Phe Leu Lys Ser Glu

225 230 235 240

Met Lys Ser Ser Ile Gln Gln Ala Asn Ser Ala Pro Val Pro Val Ser

245 250 255

Asp Asp Gly Trp Tyr Arg Pro Arg Ser Ile Asp Glu Leu His Arg Leu

260 265 270

Phe Gln Ser Ser Ser Phe Asp Glu Asn Ser Val Lys Ile Val Ala Ser

275 280 285

Asn Thr Gly Ser Gly Val Tyr Lys Asp Gln Asp Leu Tyr Asp Lys Tyr

290 295 300

Ile Asp Ile Lys Gly Ile Pro Glu Leu Ser Val Ile Asn Arg Asn Asp

305 310 315 320

Lys Gly Ile Glu Leu Gly Ser Val Val Ser Ile Ser Lys Ala Ile Glu

325 330 335

Val Leu Ser Asp Gly Asn Leu Val Phe Arg Lys Ile Ala Gly His Leu

340 345 350

Asn Lys Val Ala Ser Pro Phe Val Arg Asn Thr Ala Thr Ile Gly Gly

355 360 365

Asn Ile Val Met Ala Gln Arg Leu Pro Phe Ala Ser Asp Ile Ala Thr

370 375 380

Ile Leu Leu Ala Ala Gly Ser Thr Val Thr Ile Gln Val Ala Ser Lys

385 390 395 400

Arg Leu Cys Phe Thr Leu Glu Glu Phe Leu Gln Gln Pro Pro Cys Asp

405 410 415

Ser Arg Thr Leu Leu Leu Ser Ile Phe Ile Pro Glu Trp Gly Ser Asn

420 425 430

Asp Val Thr Phe Glu Thr Phe Arg Ala Ala Pro Arg Pro Leu Gly Asn

435 440 445

Ala Val Ser Tyr Val Asn Ser Ala Phe Leu Ala Arg Thr Ser Leu Asp

450 455 460

Ala Ala Ser Lys Asp His Leu Ile Glu Asp Ile Cys Leu Ala Phe Gly

465 470 475 480

Ala Tyr Gly Ala Asp His Ala Ile Arg Ala Arg Lys Val Glu Asp Tyr

485 490 495

Leu Lys Gly Lys Thr Val Ser Ser Ser Val Ile Leu Glu Ala Val Arg

500 505 510

Leu Leu Lys Gly Ser Ile Lys Pro Ser Glu Gly Ser Thr His Pro Glu

515 520 525

Tyr Arg Ile Ser Leu Ala Val Ser Phe Leu Phe Thr Phe Leu Ser Ser

530 535 540

Leu Ala Asn Ser Leu Asn Glu Ser Ala Lys Val Ser Gly Thr Asn Glu

545 550 555 560

His Ser Pro Glu Lys Gln Leu Lys Leu Asp Ile Asn Asp Leu Pro Ile

565 570 575

Arg Ser Arg Gln Glu Ile Phe Phe Thr Asp Ala Tyr Lys Pro Val Gly

580 585 590

Lys Ala Ile Lys Lys Ala Gly Val Glu Ile Gln Ala Ser Gly Glu Ala

595 600 605

Val Tyr Val Asp Asp Ile Pro Ala Pro Lys Asp Cys Leu Tyr Gly Ala

610 615 620

Phe Ile Tyr Ser Thr His Pro His Ala His Val Lys Ser Ile Asn Phe

625 630 635 640

Lys Pro Ser Leu Ala Ser Gln Lys Ile Ile Thr Val Ile Thr Ala Lys

645 650 655

Asp Ile Pro Ser Gly Gly Gln Asn Val Gly Tyr Ser Phe Pro Met Ile

660 665 670

Gly Glu Glu Ala Leu Phe Ala Asp Pro Val Ala Glu Phe Ala Gly Gln

675 680 685

Asn Ile Gly Val Val Ile Ala Gln Thr Gln Lys Tyr Ala Tyr Met Ala

690 695 700

Ala Lys Gln Ala Ile Ile Glu Tyr Ser Thr Glu Asn Leu Gln Pro Pro

705 710 715 720

Ile Leu Thr Ile Glu Asp Ala Ile Glu Arg Ser Ser Phe Phe Gln Thr

725 730 735

Leu Pro Phe Val Ala Pro Lys Pro Val Gly Asp Tyr Asp Lys Gly Met

740 745 750

Ser Glu Ala Asp His Lys Ile Leu Ser Ala Glu Val Lys Ile Glu Ser

755 760 765

Gln Tyr Phe Phe Tyr Met Glu Pro Gln Val Ala Leu Ala Ile Pro Asp

770 775 780

Glu Asp Asn Cys Ile Thr Ile Tyr Phe Ser Thr Gln Leu Pro Glu Ser

785 790 795 800

Thr Gln Asn Val Val Ala Lys Cys Val Gly Ile Pro Phe His Asn Val

805 810 815

Arg Val Ile Thr Arg Arg Val Gly Gly Gly Phe Gly Gly Lys Ala Leu

820 825 830

Lys Ser Met His Val Ala Cys Ala Cys Ala Val Ala Ala Leu Lys Leu

835 840 845

Gln Arg Pro Val Arg Met Tyr Leu Asp Arg Lys Thr Asp Met Ile Met

850 855 860

Ala Gly Gly Arg His Pro Met Lys Val Lys Tyr Ser Val Gly Phe Lys

865 870 875 880

Ser Asn Gly Lys Ile Thr Ala Leu His Leu Asp Leu Gly Ile Asn Gly

885 890 895

Gly Ile Ser Pro Asp Met Ser Pro Met Ile Ala Ala Pro Val Ile Gly

900 905 910

Ser Leu Lys Lys Tyr Asn Trp Gly Asn Leu Ala Phe Asp Thr Lys Val

915 920 925

Cys Lys Thr Asn Val Ser Ser Lys Ser Ser Met Arg Ala Pro Gly Asp

930 935 940

Ala Gln Gly Ser Phe Ile Ala Glu Ala Ile Ile Glu His Val Ala Ser

945 950 955 960

Ala Leu Ser Ala Asp Thr Asn Thr Ile Arg Arg Lys Asn Leu His Asp

965 970 975

Phe Glu Ser Leu Ala Val Phe Phe Gly Asp Ser Ala Gly Glu Ala Ser

980 985 990

Thr Tyr Ser Leu Val Thr Met Phe Asp Lys Leu Ala Ser Ser Pro Glu

995 1000 1005

Tyr Gln His Arg Ala Glu Met Val Glu Gln Phe Asn Arg Ser Asn Lys

1010 1015 1020

Trp Lys Lys Arg Gly Ile Ser Cys Val Pro Val Thr Tyr Glu Val Gln

1025 1030 1035 1040

Leu Arg Pro Thr Pro Gly Lys Val Ser Ile Met Asn Asp Gly Ser Ile

1045 1050 1055

Ala Val Glu Val Gly Gly Val Glu Leu Gly Gln Gly Leu Trp Thr Lys

1060 1065 1070

Val Lys Gln Met Thr Ala Phe Gly Leu Gly Gln Leu Cys Pro Gly Gly

1075 1080 1085

Gly Glu Ser Leu Leu Asp Lys Val Arg Val Ile Gln Ala Asp Thr Leu

1090 1095 1100

Ser Met Ile Gln Gly Gly Val Thr Gly Gly Ser Thr Thr Ser Glu Thr

1105 1110 1115 1120

Ser Cys Glu Ala Val Arg Lys Ser Cys Val Ala Leu Val Glu Ser Leu

1125 1130 1135

Lys Pro Ile Lys Glu Asn Leu Glu Ala Lys Thr Gly Thr Val Glu Trp

1140 1145 1150

Ser Ala Leu Ile Ala Gln Ala Ser Met Ala Ser Val Asn Leu Ser Ala

1155 1160 1165

His Ala Tyr Trp Thr Pro Asp Pro Thr Phe Thr Ser Tyr Leu Asn Tyr

1170 1175 1180

Gly Ala Gly Thr Ser Glu Val Glu Ile Asp Val Leu Thr Gly Ala Thr

1185 1190 1195 1200

Thr Ile Leu Arg Ser Asp Leu Val Tyr Asp Cys Gly Gln Ser Leu Asn

1205 1210 1215

Pro Ala Val Asp Leu Gly Gln Val Glu Gly Ala Phe Val Gln Gly Val

1220 1225 1230

Gly Phe Phe Thr Asn Glu Glu Tyr Ala Thr Asn Ser Asp Gly Leu Val

1235 1240 1245

Ile His Asp Gly Thr Trp Thr Tyr Lys Ile Pro Thr Val Asp Thr Ile

1250 1255 1260

Pro Lys Gln Phe Asn Val Glu Leu Ile Asn Ser Ala Arg Asp Gln Lys

1265 1270 1275 1280

Arg Val Leu Ser Ser Lys Ala Ser Gly Glu Pro Pro Leu Leu Leu Ala

1285 1290 1295

Ser Ser Val His Cys Ala Met Arg Glu Ala Ile Arg Ala Ala Arg Lys

1300 1305 1310

Glu Phe Ser Val Cys Thr Gly Pro Ala Asn Ser Ala Ile Thr Phe Gln

1315 1320 1325

Met Asp Val Pro Ala Thr Met Pro Val Val Lys Glu Leu Cys Gly Leu

1330 1335 1340

Asp Val Val Glu Arg Tyr Leu Glu Ser Val Ser Ala Ala Ser Pro Thr

1345

Asn Thr Ala Lys Ala

SEQ ID NO: 4

SEQUENCE LENGTH: 4,359

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays L.*)

STRAIN: cultivar: Golden Cross Bantam 70

FEATURES OF SEQUENCE:

KEY: CDS

LOCATION: 91..4138 (including termination codon)

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION

```
CCG GCT CTC TCG GTG CAG ACG TCC GGG ACT AGT ACG TGG ATC GGG CCG     48

GGG GCA ACT CGA GTC GTC AAG AAG GCT GCT ACC TGC TAG AGG ATG GAG     96

ATG GGG AAG GCG GCG GCG GTG GTG CTG GCG GTG AAC GGC AAG CGG TAC    144

GAG GCC GCC GGC GTG GAC CCG TCG ACG ACG CTG CTG GAG TTC CTG CGC    192

ACC CAC ACG CCC GTC AGG GGG CCC AAG CTC GGC TGC GGC GAA GGT GGC    240

TGC GGT GCA TGC GTT GTG CTT GTC TCG AAG TAC GAC CCA GCC ACC GAC    288

GAG GTG ACC GAG TTC TCA GCG AGC TCC TGC CTG ACG CTG CTC CAT AGC    336

GTG GAC CGC TGC TCG GTG ACC ACC AGC GAG GGC ATT GGC AAC ACC AAG    384

GAT GGC TAC CAC CCT GTG CAG CAG CGC CTC TCC GGC TTC CAC GCC TCC    432

CAG TGC GGT TTC TGC ACG CCC GGC ATG TGC ATG TCC ATC TTC TCT GCG    480
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CTT GTC AAA GCC GAC AAG GCG GCC AAC CGG CCA GCC CCA CCG GCC GGC 528

TTC TCC AAG CTC ACT TCC TCG GAG GCT GAG AAG GCT GTC TCT GGC AAC 576

CTG TGC CGC TGC ACA GGG TAC AGG CCC ATC GTC GAC GCC TGT AAG AGC 624

TTC GCA GCC GAT GTT GAT CTT GAG GAC CTG GGC CTC AAC TGC TTC TGG 672

AAG AAG GGT GAT GAG CCT GCA GAT GTC AGC AAG CTG CCA GGC TAC AAC 720

AGT GGT GAC GTC TGC ACT TTC CCT GAC TTT CTC AAA TCT GAG ATG AAG 768

TCC TCA ATT CAG CAG GCT AAC AGC GCT CCA GTT CCT GTT TCT GAC GAC 816

GGC TGG TAC CGT CCT AGG AGC ATT GAC GAG CTT CAC AGG TTG TTT CAA 864

TCT AGC TCC TTC GAT GAA AAT TCC GTG AAG ATA GTG GCT TCA AAC ACT 912

GGG TCT GGA GTG TAC AAG GAT CAG GAC CTT TAT GAC AAG TAC ATT GAC 960

ATC AAA GGA ATC CCA GAG CTT TCA GTC ATC AAC AGA AAC GAC AAA GGA 1008

ATT GAG CTT GGA TCA GTT GTG TCC ATC TCT AAA GCT ATT GAG GTG CTG 1056

TCA GAT GGA AAT CTC GTC TTC AGA AAG ATT GCT GGT CAC CTG AAC AAA 1104

GTG GCT TCA CCG TTT GTT CGG AAC ACT GCA ACC ATA GGT GGA AAC ATA 1152

GTC ATG GCA CAA AGA TTG CCA TTC GCA TCG GAC ATT GCA ACC ATA CTA 1200

CTA GCT GCA GGT TCA ACA GTC ACA ATC CAG GTG GCT TCC AAA AGG CTG 1248

TGC TTC ACT CTG GAG GAG TTC TTG CAG CAG CCT CCA TGC GAT TCT AGG 1296

ACC CTG CTG CTG AGC ATA TTT ATC CCG GAA TGG GGC TCA AAT GAT GTC 1344

ACC TTT GAG ACT TTC CGA GCA GCA CCT CGT CCA CTT GGC AAT GCT GTC 1392

TCA TAT GTC AAT TCA GCT TTC TTG GCA AGG ACT TCA TTG GAT GCA GCA 1440

TCA AAG GAC CAT CTC ATC GAG GAT ATA TGT CTG GCG TTC GGT GCT TAT 1488

GGA GCT GAT CAT GCT ATT AGA GCT AGA AAG GTT GAG GAT TAC CTG AAG 1536

GGC AAA ACA GTG AGC TCG TCT GTC ATA CTT GAA GCT GTT CGG TTG CTT 1584

AAA GGG TCT ATT AAA CCA TCA GAA GGC TCA ACA CAT CCT GAG TAT AGA 1632

ATT AGC TTG GCT GTC AGT TTC TTG TTT ACC TTC CTA TCC TCC CTT GCC 1680

AAC AGC TTG AAT GAA TCT GCA AAG GTT AGT GGT ACC AAC GAG CAC TCA 1728

CCA GAG AAG CAA CTC AAG TTG GAC ATC AAT GAT TTG CCA ATA CGA TCA 1776

AGA CAA GAA ATA TTT TTC ACT GAT GCA TAT AAG CCA GTT GGC AAA GCA 1824

ATT AAG AAA GCT GGG GTA GAG ATC CAA GCT TCA GGG GAA GCT GTG TAC 1872

GTT GAT GAT ATC CCT GCT CCC AAA GAT TGC CTC TAT GGG GCA TTT ATT 1920

TAT AGC ACA CAC CCT CAT GCA CAT GTA AAG TCA ATC AAC TTT AAA CCA 1968

TCT TTG GCT TCA CAG AAG ATC ATC ACA GTT ATC ACT GCA AAG GAT ATT 2016

CCC AGC GGT GGA CAA AAT GTT GGT TAT AGC TTC CCG ATG ATT GGA GAA 2064

GAA GCA CTT TTT GCA GAT CCA GTT GCT GAA TTT GCT GGT CAA AAT ATT 2112

GGT GTC GTG ATT GCT CAA ACA CAG AAG TAT GCC TAC ATG GCG GCA AAG 2160

CAA GCC ATC ATT GAG TAT AGC ACA GAA AAT CTG CAG CCA CCA ATT CTG 2208

ACA ATA GAA GAT GCA ATT GAA CGA AGC AGC TTC TTC CAA ACC CTC CCA 2256

TTT GTA GCT CCT AAG CCA GTT GGT GAT TAC GAC AAA GGG ATG TCT GAA 2304

GCT GAT CAC AAG ATT TTA TCG GCA GAG GTA AAA ATT GAA TCC CAA TAC 2352

TTT TTC TAC ATG GAG CCA CAA GTG GCG CTA GCT ATT CCT GAT GAA GAT 2400

AAC TGC ATA ACC ATC TAT TTT TCG ACA CAA TTA CCT GAG TCC ACA CAA 2448

AAT GTG GTT GCA AAG TGC GTT GGC ATT CCA TTT CAC AAT GTC CGT GTA 2496

ATC ACC AGA AGG GTC GGA GGA GGC TTT GGT GGA AAA GCA TTG AAA TCA 2544

ATG CAT GTT GCA TGT GCA TGT GCA GTT GCT GCA TTG AAG CTA CAA CGT 2592

CCA GTT CGG ATG TAC CTC GAT CGC AAG ACA GAC ATG ATA ATG GCA GGC 2640

GGG CGG CAT CCT ATG AAG GTG AAG TAC TCT GTT GGG TTC AAG TCA AAC 2688

GGC AAG ATC ACA GCC TTA CAT CTT GAT CTT GGG ATC AAT GGT GGA ATA 2736

TCT CCA GAT ATG AGT CCA ATG ATT GCA GCA CCT GTC ATA GGT TCT CTC 2784

AAA AAG TAC AAC TGG GGC AAT CTT GCA TTT GAC ACC AAG GTC TGC AAA 2832

ACA AAT GTC TCA TCA AAA TCG TCA ATG AGA GCT CCT GGA GAT GCG CAG 2880

GGC TCT TTC ATT GCT GAA GCC ATC ATC GAG CAT GTT GCC TCG GCA CTT 2928

TCA GCC GAC ACT AAT ACC ATA AGG AGA AAG AAC CTT CAT GAC TTT GAG 2976

AGC CTT GCA GTG TTC TTT GGA GAT AGT GCA GGT GAA GCT TCT ACA TAC 3024

AGC CTT GTC ACC ATG TTC GAT AAA TTG GCC TCC TCT CCA GAA TAC CAG 3072

CAC CGA GCT GAA ATG GTG GAA CAA TTC AAC CGA AGC AAC AAG TGG AAG 3120

AAG CGT GGC ATT TCT TGT GTG CCT GTA ACA TAT GAG GTG CAG CTT CGG 3168

CCA ACT CCA GGA AAG GTG TCT ATC ATG AAT GAT GGT TCC ATT GCT GTT 3216

GAG GTT GGA GGG GTT GAG CTA GGC CAA GGG TTG TGG ACA AAA GTG AAG 3264

CAG ATG ACG GCA TTC GGA CTA GGA CAG CTG TGT CCT GGC GGC GGT GAA 3312

AGC CTT CTA GAC AAG GTG CGG GTC ATC CAG GCC GAC ACA TTG AGC ATG 3360

ATC CAA GGA GGG GTC ACT GGT GGG AGC ACC ACT TCT GAA ACT AGC TGT 3408

GAA GCA GTT CGT AAG TCG TGT GTT GCA CTC GTC GAG AGC TTG AAG CCA 3456

ATC AAG GAG AAT CTG GAG GCT AAA ACT GGC ACA GTG GAA TGG AGT GCC 3504

TTG ATT GCA CAG GCA AGT ATG GCG AGC GTT AAC TTA TCG GCA CAT GCA 3552

TAC TGG ACC CCT GAT CCA ACT TTC ACA AGC TAT TTG AAC TAC GGA GCC 3600

GGC ACT AGC GAG GTG GAA ATT GAT GTC CTG ACA GGA GCA ACA ACA ATT 3648

CTA AGG AGT GAC CTT GTC TAC GAT TGC GGG CAA AGC TTG AAC CCT GCT 3696

GTC GAT TTG GGG CAG GTG GAA GGT GCA TTC GTA CAA GGA GTA GGC TTC 3744

TTC ACA AAC GAG GAG TAC GCA ACC AAC TCT GAC GGG TTG GTC ATC CAC 3792

GAT GGC ACA TGG ACG TAC AAG ATC CCC ACG GTC GAC ACC ATC CCA AAG 3840

CAG TTC AAC GTT GAG CTG ATC AAC AGC GCC CGT GAC CAG AAG CGC GTC 3888

CTC TCT TCC AAA GCA TCG GGC GAG CCT CCG CTT CTC CTA GCT TCC TCT 3936

GTG CAC TGC GCA ATG AGG GAG GCC ATC AGG GCC GCC AGG AAA GAA TTC 3984

TCG GTC TGC ACT GGT CCA GCG AAC TCC GCC ATC ACG TTC CAG ATG GAC 4032

GTG CCG GCA ACG ATG CCT GTC GTC AAG GAG CTC TGC GGC CTG GAT GTC 4080

GTT GAG AGG TAC CTG GAG AGC GTG TCG GCT GCC AGC CCA ACA AAC ACC 4128

GCT AAA GCA TAG ATC CAG TAG GCG CTC TAT CCA TGG TGT GAT GGC TTA 4176

ATC AAT CTG TAA AAC ACT AAG CGG CGT GAC ATG CCG AGC TTT CAG TGT 4224

TAG CTA TGA TGT ACA GAA GAA GAG GTA CCA ATG GCG AGT TGT GGC CAT 4272

GCG AAT CAG GAG TCA TGA ACC ATT GAG GGG GGA AAT AAA GTA AAT AAG 4320

TGT TGC GCC GGC GAA AAA AAA AAA AAA AAA AAA AAA AAA 4359

CLAIMS

1. An aldehyde oxidase gene which is a 4.4 Kbp gene obtainable from a plant and which encodes an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to a carboxylic acid.

2. The aldehyde oxidase gene according to claim 1, wherein the aldehyde compound is indoleacetaldehyde and the carboxylic acid is indoleacetic acid.

3. The aldehyde oxidase gene according to claim 1 or 2 which is derived from maize plant (*Zea mays L.*).

4. The aldehyde oxidase gene according to claim 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1.

5. The aldehyde oxidase gene according to claim 4 which has a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120).

6. The aldehyde oxidase gene according to claim 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 3.

7. The aldehyde oxidase gene according to claim 6 which has a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138).

8. A plasmid comprising the aldehyde oxidase gene according to claim 1, 2, 3, 4, 5, 6 or 7.

9. A transformant transformed by introducing the plasmid according to claim 8 into a host cell.

10. The transformant according to claim 9, wherein the host cell is a microorganism.

11. The transformant according to claim 9, wherein the host cell is a plant.

12. A process for constructing an expression plasmid which comprises ligating (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene according to claim 1, 2, 3, 4, 5, 6 or 7 and (3) a terminator capable of functioning in a plant in a functional manner and in the said order described above.

13. An expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene according to claim 1, 2, 3, 4, 5, 6 or 7 and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above.

14. A process for controlling production of an aldehyde oxidase in a transformant which comprises introducing, into a host cell, an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above to transform said host cell.

15. The process according to claim 14, wherein the aldehyde oxidase gene is derived from a plant and the host cell is a plant cell.

16. The process according to claim 13, wherein the expression plasmid is the expression plasmid according to claim 13.

ABSTRACT

There is provided an aldehyde oxidase gene which is a 4.4 kbp gene obtainable from a plant and which encodes an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to a carboxylic acid and utilization thereof.